

Formation of Moniliformin by *Fusarium sporotrichioides* and *Fusarium culmorum*

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Two strains of *Fusarium sporotrichioides* and one strain of *F. culmorum* were shown to produce the mycotoxin moniliformin in rice culture. Identification was by reverse-phase liquid chromatography, thin-layer chromatography, and mass spectrometry.

Moniliformin is the sodium or potassium salt of 1-hydroxycyclobut-1-ene-3,4-dione (16, 18) and was discovered as a mycotoxin from *Fusarium moniliforme* by Cole et al. (8). Its natural occurrence in corn grown in Transkei (20, 21) and the Federal Republic of Germany (19) has been reported. The oral 50% lethal doses of moniliformin in cockerels, chickens, and ducklings are in the range of 3.7 to 5.4 mg/kg of body weight (5, 8, 10), whereas in rats and mice they are from 42 to 50 mg/kg of body weight (6, 10). The following *Fusarium* species are also producers of moniliformin: *F. moniliforme* var. *subglutinans* (*F. subglutinans*), *F. proliferatum*, *F. anthophilum*, *F. graminearum*, *F. avenaceum*, *F. acuminatum*, *F. concolor*, *F. equiseti*, *F. oxysporum*, *F. semitectum*, and *F. fusarioides* (*F. chlamydosporum*) (7, 11-13, 15). The last-named species is the only member of the section *Sporotrichiella* known to produce moniliformin. We report here evidence for the formation of moniliformin by two additional *Fusarium* species, *F. sporotrichioides* and *F. culmorum*, both of which are important mycotoxigenic fungi occurring on grains and other host plants (12).

F. sporotrichioides 888 and 951 were isolated from *Baccharis coridifolia* (T. Kommedahl, H. K. Abbas, C. J. Mirocha, G. A. Bean, B. B. Jarvis, and M.-D. Guo, Phytopathology, in press), and *F. culmorum* HM-8 was obtained from J. Lacey (Rothamsted Experimental Station, Harpenden, United Kingdom), who originally isolated it from wheat (2). Fungi were identified by the method of Nelson et al. (14) and cultured on long-grain polished rice for 2 weeks at 25 to 27°C followed by 2 weeks at 10°C (2). The air-dried rice culture was ground, and 0.5 g was extracted with 95% acetonitrile-5% water, defatted with *n*-hexane, and cleaned up on small C₁₈ (Baker-10; J. T. Baker Chemical Co., Phillipsburg, N.J.) and neutral alumina columns successively for moniliformin determination by reverse-phase liquid chromatography (LC) (P. M. Scott and G. A. Lawrence, Abstr. 100th Annu. Int. Meet. Assoc. Off. Anal. Chem. 1986; J. Assoc. Off. Anal. Chem., in press). The LC mobile phases were 10 or 15% methanol and 10 or 15% acetonitrile in aqueous ion pair reagent (5 mM tetrabutylammonium hydroxide, 11 mM KH₂PO₄), used with a 5- μ m octadecylsilane column (4.6-mm internal diameter by 250 mm). Moniliformin was detected (at retention times of 8 to 14 min) by UV absorption at 229 and 254 nm. Control rice samples (Table 1) were also analyzed for moniliformin.

Confirmation of identity of moniliformin in rice culture extracts was carried out by thin-layer chromatography (17) with aqueous 3-methyl-2-benzothiazolinone hydrazone hydrochloride as spray reagent (9). Furthermore, extracts in the LC mobile phase (0.5 ml) were acidified with 2 drops of concentrated HCl or concentrated H₂SO₄ and extracted two times with 0.5 ml of methylene chloride, and the solvent was evaporated for confirmation of the free acid (3) by negative chemical ionization mass spectrometry. The instrument used was a model 7070 EQ mass spectrometer (VG Analytical Ltd., Manchester, United Kingdom) (operated in the conventional sector only), with methane or isobutane as reagent gas and sample introduction by a direct probe heated from the ambient temperature to 250°C. Rice cultures were also analyzed for fusarin C, another mycotoxin from *F. moniliforme*, by the LC method of Scott et al. (17).

Results of analyses of rice cultures by LC show formation of moniliformin by *F. sporotrichioides* and *F. culmorum* at concentrations of 2.7 to 182 μ g/ml (Table 1). For *F. sporotrichioides* 951, which formed the most moniliformin, only one other minor UV-absorbing peak was observed. These LC determinations were confirmed by thin-layer chromatography. The characteristic moniliformin spot at the same *R_f* (0.38) as standard was clearly visible when 1- to 2-mg equivalent amounts of *F. sporotrichioides* 951 and *F. culmorum* HM-8 powdered rice cultures were developed on the thin-layer chromatography plate but was only barely detectable from a 6-mg equivalent of *F. sporotrichioides* 888; rice controls were negative. The methane negative chemical ionization mass spectrum of standard moniliformin free acid (3) had ions at *m/z* 97 (base peak = 100%), 98 (13%), and 195 (8%), the latter indicating dimerization. The negative chemical ionization mass spectra of acidified extracts showed a prominent signal at *m/z* 97 for *F. sporotrichioides* 951 and 888 and *F. culmorum* HM-8 and also for the autoclaved rice control sampled from the top of the package but not for the two other extracts of rice controls or for the solvent blank, thus qualitatively confirming the LC results.

This is the first report of moniliformin production by *F. sporotrichioides* and *F. culmorum*. The former species has not previously been investigated for this mycotoxin. It will be interesting to determine whether moniliformin can be formed in *Baccharis* species by *F. sporotrichioides*. Fourteen strains of *F. culmorum* grown on corn have been assayed for moniliformin by two different laboratories, with negative results (4, 11). It is possible, however, that corn is

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TABLE 1. Concentrations of moniliformin in rice cultures of *F. sporotrichioides* and *F. culmorum* determined by LC

Sample	Concn of moniliformin as potassium salt ($\mu\text{g/g}$) with:			
	10 or 15% Methanol		10 or 15% Acetonitrile	
	229 nm	254 nm	229 nm	254 nm
<i>F. sporotrichioides</i> 888	2.7	4.0	8.0	5.7
<i>F. sporotrichioides</i> 951	177	164	182	166
<i>F. culmorum</i> HM-8	10.3	11.7	12.5	11.4
Rice control, autoclaved ^a	<0.02 ^b	<0.12 ^b	0.08	tr ^b
Rice control, autoclaved ^c	<0.02 ^b	<0.12 ^b	<0.01	<0.06 ^b
Rice control, not autoclaved ^d	<0.02 ^b	<0.12 ^b	<0.01	<0.06 ^b

^a From University of Minnesota, unmixed, subsample from top of package.

^b Extract 5 weeks old (stored at -4°C).

^c From University of Minnesota, subsample from mixed sample.

^d Sample obtained in Ottawa.

not as good a culture medium as rice for screening *Fusarium* strains for moniliformin (1).

We also identified fusarin C, a mutagenic metabolite of *F. moniliforme* (17), in the *F. culmorum* HM-8 rice culture. The estimated concentration was $7.1 \mu\text{g/g}$, and the LC peak disappeared on irradiation of the extract solution under fluorescent light for 10 min, as did standard fusarin C. The two *F. sporotrichioides* cultures were negative for fusarin C. *F. culmorum* is already known as a producer of fusarin C (J. M. Farber and G. M. Sanders, J. Agric. Food Chem., in press).

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